

Pathogenic Bacteria Populations in Inoculated Fresh Pork after Chilled Storage and Simulated Consumer Abuse

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SUMMARY

Microbiological contamination of animal carcasses is an undesirable but unavoidable result of slaughter procedures and, if followed by mishandling and abuse of fresh meat products, which can occur at any point in the food chain (e.g., processing, at supermarkets/restaurants or in the home) it may lead to possible proliferation of any pathogens present. A study was designed to investigate the responses of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Yersinia enterocolitica* and *Listeria monocytogenes* after cold (32°F) storage under vacuum and subsequent aerobic temperature abuse (60, 70 or 80°F) in inoculated ground pork and pork loin chops. The results verify that temperature abuse may promote proliferation of some pathogens and demonstrate the importance of consumer education in safe food handling practices.

Key words: pork, contamination, retail, consumer

INTRODUCTION

Public awareness about food safety issues has increased during the last decade (McIlveen et al., 1999), and incidence of food-borne diseases continues to increase resulting in substantial losses to the food industry and consumers. Worsfold and Griffith (1997), in their evaluation of food safety behavior of consumers, reported that temperature abuse during food transport and storage was exhibited by more than 40% of the people. Consumer education is a key component of pathogen reduction strategies because a significant number of food-borne illness outbreaks are caused in part by simple food mishandling practices (Tietjen and Fung, 1995). The most common

mishandling practices are inadequate cooking as well as prolonged cooling or thawing at room temperature. Altekruze et al. (1995) conducted a study to assess consumer knowledge of specific microbial pathogens in food, their knowledge of certain food safety principles and their typical food handling practices. One-third of the respondents reported unsafe food handling practices such as not washing their hands or not taking precautions to prevent cross-contamination from raw meat. A similar study, conducted by Woodburn and Raab (1997), involved a telephone survey of food preparers and revealed that knowledge about food-borne illnesses was greater than in previous studies, though only 60% recognized the role of thorough cooking.

The safety and shelf-life of meat and meat products are very much dependent on the initial microbial contamination, as well as use of good manufacturing practices, proper packaging and appropriate storage temperature of the finished product (Podolak et al., 1996; Van Netten et al., 1997). Conventional wisdom of decades ago held that properly refrigerated foods would remain safe because it was thought that pathogenic bacteria could not grow at refrigeration temperatures (Marth, 1998), but we now know that muscle foods may promote the proliferation of a wide range of microorganisms, including psychrotrophs, especially when such foods are not preserved or stored properly. Food professionals should try to ensure the safety of fresh meat by controlling contamination and microbial proliferation; however, we must not overlook the responsibility the consumer has to ensure the safety of the products they consume. The objective of this study was to evaluate changes in bacterial pathogens inoculated in fresh pork chops or ground pork and abused during simulated consumer handling following vacuum packaged storage.

Materials & Methods

Pork center-cut loins were sliced to a 2.5 cm thickness with a meat slicer (Hobart Mfg. Co., Troy, OH) then cut

into 4 cm wide by 8 cm long pieces and placed into sterile plastic bags. Pork trim was processed into ground pork using a 1/8 inch plate grinder (Hobart Mfg. Co., Troy, OH) and divided into 100 g portions in individual sterile vacuum bags. The inoculated pathogens included *L. monocytogenes* (four pork variety meat isolates, LCDC 81861 and Scott A), *Salmonella* (group B, S. Enteritidis, four pork carcass isolates and two pork variety meat isolates), *Y. enterocolitica* (one pork variety meat isolate and ATCC 51871), *C. jejuni* (five pork variety meat isolates) and *E. coli* O157:H7 (ATCC 43895, 43888, 43889, 43890, 51657, 51658). Each pathogen strain was incubated individually in tryptic soy broth with 0.6% yeast extract at 37°C for 24 h with the exception of *Campylobacter*, which was incubated at 42°C in a microaerophilic environment. The pathogen strains were then combined in a sterile container and serially diluted in 9 ml Butterfield's Phosphate Buffer to obtain the desired inoculum level for inoculation of the product. Each sample was inoculated with approximately 10³- 10⁵ CFU/cm² (chops) or CFU/g (ground) of each of the pathogens (each sample was inoculated with one pathogen). After inoculation, each sample was placed in a bag and a vacuum (-85 bar) was drawn using a commercial vacuum packaging machine (Hollymatic Co. Countryside, IL).

Samples were then stored at 32°F for 18 d (ground pork) and 20 days (pork chops). Following storage, samples were aseptically transferred into styrofoam trays lined with absorbent pads and wrapped with a polyvinyl-chloride film simulating retail tray packages. One set of samples was held for 24 h at 40°F (to simulate retail display) and the other set was divided equally among incubators calibrated at 60, 70 or 80°F. For each organism, samples (one per treatment in each of three replicates) were analyzed immediately after inoculation but before storage (32°F), following storage but before temperature abuse, and after 3 and 6 h of temperature abuse at 60, 70 or 80°F. For analysis,

100 ml of Butterfield's Phosphate Buffer was added to each sample and the samples were shaken in a 30 cm arc 30 times (chops) or stomached (IUL Instruments, Barcelona, Spain) for 2 min (ground). The ground sample was further diluted by adding 10 g of the previously generated slurry to 40 ml of sterile Butterfield's Phosphate Buffer and stomached for 2 min to yield the initial 1:10 dilution. Each sample was then serially diluted in 9 ml sterile Butterfield's Phosphate Buffer, and plated on a general growth medium (TSA), lactic acid bacteria medium (MRS), 3M™ Total Coliform Count Petrifilm™, and selective agar media appropriate for each organism by depositing 0.1 ml of three consecutive dilutions on duplicate plates and spreading the sample with a sterile, bent glass rod. Plates were then inverted and incubated at 25 (aerobically), 35 (aerobically) or 42°C (microaerophilic environment) depending on the temperature appropriate for the organism. Colonies were counted after 24-48 h and results were expressed as log CFU/cm² (chops) or log CFU/g (ground) for calculation of the means and standard deviations.

RESULTS

In the pork chop samples analyzed immediately following 20 d of storage (32°F), only samples inoculated with *C. jejuni* showed a decline in counts, while samples inoculated with *E. coli* O157:H7, *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* had relatively constant bacterial populations (Table 1). Bacterial counts from samples inoculated with *L. monocytogenes* and *Salmonella* showed only small fluctuations during storage at various temperatures. Samples inoculated with the other pathogens and stored at 60°F for 3 h showed an initial decline in bacterial populations compared to samples analyzed prior to storage (20 d, 32°F). This decline was followed by a 0.1-0.5 log CFU/cm² increase at 6 h indicating the initial temperature change may lead to organism stress and injury followed by repair and proliferation of the pathogens. *Campylobacter jejuni*, showing the most dramatic decrease

immediately following 20 d (32°F), increased approximately 1.0 log CFU/cm² at all three temperatures at 6 h of storage and showed the greatest amount of variation of all the pathogens. After an initial decline in counts on samples inoculated with *E. coli* O157:H7 showed an increase at 6 h (60, 70 and 80°F). *Yersinia enterocolitica* populations declined after 3 h at 60°F and 6 h at 70°F while showing minimal growth after 6 h at 60 and 80°F and drastic growth after 3 h at 70°F.

Simulated retail display (24 h, 40°F) seemed to improve the chances of pathogen survival and growth. The initial decline in bacterial numbers was noted in these samples also; however, in most cases, retail storage samples showed higher populations at 3 h storage (60, 70 and 80°F). After a 0.8-0.9 log CFU/cm² increase at 3 h (60, 70 and 80°F), populations in samples inoculated with *C. jejuni* showed inconsequential differences at 6 h (60, 70 and 80°F). Populations from samples inoculated with *Salmonella* changed very little throughout the study, while those from samples inoculated with *Y. enterocolitica* showed increases of 1.8 and 2.3 log CFU/cm² at 3 h (60 and 80°F), respectively. At 6 h (60 and 80°F), however, these populations declined by 1.7 and 1.8 log CFU/cm², respectively. Bacterial populations for samples inoculated with *L. monocytogenes* and *E. coli* O157:H7 populations showed similar trends in that growth was demonstrated at 3 h (60, 70 and 80°F and 6 h (70 and 80°F) whereas there was a slight decline at 6 h (60°F).

In ground pork samples analyzed immediately following 18 d of storage (32°F), there was a decline in detectable bacterial populations in samples inoculated with *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* (Table 2), though not as drastic as that observed for the pork chops (Table 1). The bacterial populations in these samples did not reach the levels achieved in the samples analyzed prior to 18 d of storage (32°F); however, the counts at

0 h were very similar to those for samples analyzed at 3 and 6 h (60, 70 and 80°F). Populations for samples inoculated with *C. jejuni* after 3 h (60, 70 and 80°F) of storage remained similar to those at 0 h (4.4-4.6 log CFU/g), but declined in numbers at 6 h (60, 70 and 80°F) by 0.5-0.7 log CFU/g. Populations on samples inoculated with *L. monocytogenes* remained fairly stable throughout storage, with little growth occurring at 3 and 6 h at 80°F. The most dramatic reduction (1.2 log CFU/g) in counts at 18 d (32°F) was observed in samples inoculated with *E. coli* O157:H7, which also indicated growth/recovery of 0.3-0.9 log CFU/g at 3 and 6 h (60, 70 and 80°F), with the greatest increase at 80°F. Inasmuch as *Salmonella* inoculated samples, populations showed inconsequential variation, while in *Y. enterocolitica* inoculated samples bacterial populations were favored in this environment; this was the only pathogen to proliferate (>1.0 log CFU/g) during storage (18 d, 32°F) with increases of 0.2-0.6 log CFU/g at 6 h (70 and 80°F). Simulated retail display of the ground pork samples for 24 h at 40°F did not seem to affect pathogen growth as compared to that for samples that were not displayed.

IMPLICATION

These results verify that temperature abuse may promote proliferation of some pathogens and the results should be useful in risk assessment studies. This study also demonstrates the importance of consumer education with respect to safe food handling practices, and the need for minimizing initial contamination levels of fresh meat.

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Table 1 Mean [log CFU/cm² (SD)] bacterial counts determined on selective agar media by plating samples of pork chops inoculated with *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., or *Yersinia enterocolitica* and subjected to aerobic temperature abuse (60, 70 or 80°F) for 3 or 6 h following 20 days of vacuum storage at 32°F and display at 40°F for 0 or 24 h.

| Storage at 32°F (days) | Simulated display at 40°F (hours) | Abusive temperature (°F) and time (h) | N | Bacterial Counts [log CFU/cm ² (SD)] | | | | | |
|------------------------|-----------------------------------|---------------------------------------|---|---|--|--|--------------------------|--------------------------------------|-----------|
| | | | | <i>Campylobacter jejuni</i> (MCCDA) | <i>Escherichia coli</i> O157:H7 (SMAC) | <i>Listeria monocytogenes</i> (PALCAM) | <i>Salmonella</i> (XLT4) | <i>Yersinia enterocolitica</i> (CIN) | |
| 0 | - | 0 | 6 | 4.1 (1.8) | 5.0 (1.1) | 5.1 (0.1) | 4.5 (1.2) | 4.5 (0.4) | |
| 20 | 0 | - | 3 | 3.3 (2.1) | 5.2 (0.2) | 4.4 (0.3) | 4.5 (0.4) | 4.9 (0.8) | |
| 20 | 0 | 60 | 3 | 2.2 (2.3) | 4.7 (0.3) | 4.5 (0.3) | 4.7 (0.4) | 4.0 (0.3) | |
| 20 | 0 | 60 | 6 | 3 | 4.4 (0.4) | 5.4 (0.9) | 4.6 (0.6) | 4.7 (0.1) | 5.0 (1.5) |
| 20 | 0 | 70 | 3 | 3 | 3.4 (2.2) | 4.7 (0.5) | 4.2 (0.4) | 4.5 (0.3) | 5.8 (1.6) |
| 20 | 0 | 70 | 6 | 3 | 4.5 (0.3) | 5.1 (0.6) | 4.9 (1.4) | 4.7 (0.3) | 4.3 (1.0) |
| 20 | 0 | 80 | 3 | 3 | 3.4 (2.2) | 4.8 (0.1) | 4.7 (1.2) | 4.4 (0.1) | 5.3 (1.3) |
| 20 | 0 | 80 | 6 | 3 | 4.3 (0.3) | 5.2 (0.4) | 4.4 (1.3) | 4.7 (0.6) | 5.1 (0.5) |
| 20 | 24 | - | 0 | 3 | 3.6 (2.4) | 4.8 (0.2) | 4.1 (0.4) | 4.3 (0.2) | 4.5 (2.2) |
| 20 | 24 | 60 | 3 | 3 | 4.4 (0.4) | 5.6 (0.8) | 5.1 (1.3) | 4.7 (0.7) | 6.3 (0.6) |
| 20 | 24 | 60 | 6 | 3 | 4.2 (0.3) | 5.0 (0.6) | 4.2 (0.2) | 3.6 (0.7) | 4.6 (0.6) |
| 20 | 24 | 70 | 3 | 3 | 4.4 (0.3) | 4.8 (0.1) | 4.7 (1.0) | 4.4 (0.2) | 4.4 (1.1) |
| 20 | 24 | 70 | 6 | 3 | 4.3 (0.3) | 5.0 (0.5) | 5.1 (1.5) | 4.3 (0.6) | 4.5 (2.0) |
| 20 | 24 | 80 | 3 | 3 | 4.5 (0.2) | 5.1 (0.5) | 4.5 (0.8) | 4.5 (0.9) | 6.8 (1.4) |
| 20 | 24 | 80 | 6 | 3 | 4.6 (0.4) | 5.4 (1.0) | 5.1 (1.2) | 4.0 (0.4) | 4.9 (1.7) |

MCCDA = Modified Campylobacter Charcoal Differential Agar, SMAC = Sorbitol MacConkey Agar, XLT4 = Xylose Lysine Tergitol 4 Agar, CIN = Yersinia Selective Agar.

Table 2 Mean [log CFU/cm² (SD)] bacterial counts determined on selective agar media by plating samples of ground pork inoculated with *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., or *Yersinia enterocolitica* and subjected to aerobic temperature abuse (60, 70 or 80°F) for 3 or 6 h following 20 days of vacuum storage at 32°F and display at 40°F for 0 or 24 h.

| Storage at 32°F (days) | Simulated display at 40°F (hours) | Abusive temperature and time (°F) (h) | N | Bacterial Counts [log CFU/cm ² (SD)] | | | | |
|------------------------|-----------------------------------|---------------------------------------|---|---|--|--|--------------------------|--------------------------------------|
| | | | | <i>Campylobacter jejuni</i> (MCCDA) | <i>Escherichia coli</i> O157:H7 (SMAC) | <i>Listeria monocytogenes</i> (PALCAM) | <i>Salmonella</i> (XLT4) | <i>Yersinia enterocolitica</i> (CIN) |
| 0 | - | 0 | 6 | 5.1 (0.3) | 4.8 (0.5) | 4.8 (0.8) | 4.6 (0.6) | 4.4 (0.6) |
| 18 | 0 | 0 | 3 | 4.5 (0.3) | 4.2 (0.4) | 4.3 (0.7) | 3.7 (0.5) | 5.5 (1.1) |
| 18 | 0 | 60 | 3 | 4.6 (0.4) | 4.6 (1.0) | 4.4 (0.8) | 3.7 (0.7) | 5.3 (1.4) |
| 18 | 0 | 60 | 6 | 4.0 (0.9) | 4.4 (0.8) | 4.6 (1.0) | 3.6 (0.5) | 5.5 (1.0) |
| 18 | 0 | 70 | 3 | 4.6 (0.5) | 4.5 (0.9) | 4.4 (1.0) | 3.9 (0.4) | 5.5 (1.0) |
| 18 | 0 | 70 | 6 | 3.9 (0.8) | 4.5 (0.6) | 4.6 (1.0) | 3.7 (0.5) | 5.7 (1.1) |
| 18 | 0 | 80 | 3 | 4.4 (0.3) | 4.6 (1.1) | 5.0 (1.2) | 3.8 (0.6) | 5.4 (1.4) |
| 18 | 0 | 80 | 6 | 3.9 (1.4) | 4.9 (0.9) | 4.8 (1.2) | 4.1 (0.6) | 6.1 (1.1) |
| 18 | 24 | - | 3 | 3.1 (1.8) | 4.2 (0.5) | 4.7 (1.1) | 3.8 (0.5) | 5.7 (1.1) |
| 18 | 24 | 60 | 3 | 4.0 (1.3) | 4.9 (0.9) | 4.8 (0.4) | 3.9 (0.6) | 5.8 (0.8) |
| 18 | 24 | 60 | 6 | 4.7 (0.5) | 4.8 (1.2) | 4.7 (0.9) | 3.6 (0.6) | 5.9 (0.5) |
| 18 | 24 | 70 | 3 | 4.5 (0.1) | 4.6 (0.8) | 4.9 (1.3) | 3.8 (0.5) | 5.6 (1.0) |
| 18 | 24 | 70 | 6 | 4.1 (0.4) | 5.4 (1.0) | 5.3 (0.7) | 3.8 (0.6) | 6.3 (0.7) |
| 18 | 24 | 80 | 3 | 4.5 (0.6) | 5.3 (0.6) | 5.1 (0.6) | 3.8 (0.4) | 5.9 (0.9) |
| 18 | 24 | 80 | 6 | 3.7 (0.6) | 5.6 (0.8) | 5.5 (0.7) | 4.3 (0.9) | 6.5 (0.5) |

MCCDA = Modified Campylobacter Charcoal Differential Agar, SMAC = Sorbitol MacConkey Agar, XLT4 = Xylose Lysine Tergitol 4 Agar, CIN = Yersinia Selective Agar.